CONTRIBUTIONS OF CONTROLLED SLOW BREATHING TO COGNITIVE FLEXIBILITY ACROSS THE LIFESPAN

A Thesis

Presented to the Faculty of the Graduate School

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In Partial Fulfillment of the Requirements for the Degree of

Master of Arts

by

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ABSTRACT

All highly developed countries around the globe are experiencing considerable increases in the proportion of aged in the population nowadays. The decline of cognition as well as physical status associated with normal aging are expected to severely impact our quality of life. Hence, it is of great importance to developing simple and effective approaches to help older adults to combat age-related changes in cognition and establish a set of lifestyle changes that would benefit the brain health across the lifespan.

To examine if controlled deep breathing can help combat age-related decline, we carried out the breathing and attention tasks among young and older adults. Our findings demonstrated that controlled slow breathing significantly increase heart rate variability of elder adults, suggesting that there appears to be potential for use of controlled slow breathing techniques as an effective noninvasive vagal nerve stimulation, especially in older adults.

BIOGRAPHICAL SKETCH

Nan Zhou was born in Shenqiu county, China, in 1991.

She received the B.A. degree in Chinese Language and Literature from the Wuhan University, China, in 2012 and the MEd. in Teaching Chinese as Foreign Language from the Zhengzhou University, China, in 2016.

In 2018, she enrolled in the Developmental Psychology, Human Development, Cornell University as a master student. Since 2018, she has been doing research in Dr. De Rosa and Dr. Anderson's Affect and Cognition Lab.

Her current research interests include affective and cognitive neuroscience, healthy aging, biological neuroscience and computational neuroscience.

To Han Wang,

My best friend,

My beloved one,

My forever supporter.

To Nan Zhou,

You are talented and smart,

You are beautiful both inside and outside,

You are the strongest person I have ever seen.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BB: Baseline breathing

SB: Sham breathing

CB: Controlled slow breathing

PI: Proactive interference

RR: Response reversal

HR: Heart rate

VT: Vagal tone

HRV: Heart rate variability

RRI: Time interval between successive ECG R-waves (RR interval, same as IBI)

IBI: Inter-beat-interval (same as RR interval)

QRS: QRS complex of electrocardiogram

ECG: Electrocardiogram

PPG: Photoplethysmogram (measurement of blood volume changes)

CNS: Central nervous system

ANS: Autonomic nervous system

SNS: Sympathetic nervous system

PNS: Parasympathetic nervous system

VNS: Vagal nerve stimulation

nVNS: Non-invasive vagal nerve stimulation

RMSSD: Root Mean Square of the Successive Differences

INTRODUCTION

It is well appreciated that brain variability – which may support adaptive cognition is constructive and important(Arieli, Sterkin, Grinvald, & Aertsen, 1996; Carandini, 2004). Growing evidence suggests that brain variability significantly decreases with advanced age and thus contribute to a decline in the cognitive mechanisms of speed, inhibitory function across the lifespan(Lövdén et al., 2013). These cognitive changes can significantly alter our quality of life, impact our communities and are associated with cognitive challenges as well(Bherer, Erickson, & Liu-Ambrose, 2013).

Among such challenges is a decline in older adults' ability to appropriately respond to dynamic environments, which is fatal to one's survival and well-being(Davis, Marra, Najafzadeh, & Liu-Ambrose, 2010; Ebert & Anderson, 2009; Emery, Hale, & Myerson, 2008; Lacreuse, Parr, Chennareddi, & Herndon, 2018; Wilson, Nusbaum, Whitney, & Hinson, 2018). To adaptively meet these varying demands, one need to adjust ways of thinking and consequently behaving, such as separating old memories from new learning(Picciotto, Higley, & Mineur, 2012), suppressing learned responses that are no longer appropriate and explore novel strategies, applying rules adaptively in diverse contexts(Carandini, 2004) which here referred as cognitive flexibility. A wealth of evidence in the literature suggests that for both humans and animals, this set of cognitive processes which is crucial for adapting to external environment is commonly decreased along with healthy aging(Armbruster-Genç, Ueltzhöffer, & Fiebach, 2016).

All highly developed countries around the globe are experiencing considerable increases in the proportion of aged in the population due to falling birth rate combined with longevity(Crimmins & Zhang, 2019). The decline of cognition as well as physical status associated with normal aging are expected to severely impact our quality of life and pose a mounting strain on the community with a high cost to affected individuals and families. Studies of body-mind relationship highlights the primary regulatory function of the vagus nerve – which is well appreciated as a key element of communication between the body and the brain, on the unconscious processes(Hays, 2019). To be more specific, the vagus nerve achieves this regulation together with the two parts of the autonomic system – the parasympathetic and sympathetic nervous system – and function synergistically to flexibly regulates our body. The interaction between the parasympathetic and sympathetic nervous system exert influences on the heart, leading to an increase or decrease of the heart rate while running or lying down. Based on this strong impact, heart rate variability (HRV), which demonstrates the brain's regulation of the heart, developed into one primary approach to quantifying vagal tone. While there is increasing evidence that vagal tone, with the index of HRV, contributes to cognitive flexibility. Previous studies have demonstrated that vagal nerve stimulation (VNS) is an already approved, effective, highly practical and successful therapy for protecting cognition due to its strong safety and tolerability profile(Howland, 2014). Several lines of evidence suggest that noninvasive vagal nerve stimulus (nVNS) like slow breathing(Ben-Tal, Shamailov, & Paton, 2012; Hirsch & Bishop, 1981; Howland, 2014) or rubbing the outer ear (Ellrich, 2011) also exert effects on the body-mind relationship, which might furthermore benefits cognition for the seniors (Kaczmarczyk, Tejera, Simon, & Heneka, 2018).

At present, a growing body of literature suggests that specific types of cognitive training exert positive changes on the brain and help sustain cognitive health, but it is unclear about the biological mechanism behind the effect(La Rue, 2010). However, the existing approach, i.e. commercial brain training program and physical exercise, is costly and often too limited due to the decrease of our physical abilities during aging(Simons et al., 2016). Hence, it is of great importance to develop simple and effective approaches to help older adults to combat age-related changes in cognition and furthermore, learn how to tailor effective and lasting tools to improve body-brain connectivity that can protect cognition, and thus establish a set of lifestyle changes that would benefit the brain health across the lifespan(Dobson & Dozois, 2019).

BACKGROUND

Body and Mind – Bidirectional Relationship

Most people are aware of the fact that our thoughts can affect the body, as one's hands get sweaty when nervous; heart races when frightened, but what about the reverse - can the physical state also influence the mind? Accumulating research is revealing that the relationship of the mind and the body state is bidirectional(Herbert & Pollatos, 2012; Huang & Galinsky, 2011). The body can also exert effects on the mind, as with eyebrows raised you may be more surprised by what you heard; just sitting up straight, you may feel more confident(Kellerman, 1992).

Cognitive Decline with Aging

The vast body of behavioral research in cognitive aging suggests that cognition declines as individuals age (Park, 2000; Skirbekk, 2004; Volkow et al., 1998), i.e., the aged brain is less efficient in terms of information processing, including slower cognitive processing, poorer inhibitory function et al (Braver et al., 2001). In comparison with the young, older participants demonstrate slower reaction times in behavioral tasks. Consistent with findings from both animal and human research (Ebert & Anderson, 2009; Merhav, Riemer, & Wolbers, 2019), studies in our laboratory have found that older rats fail to resolve proactive interference over time compared to young, suggesting that the PI resolution task successfully taps into age-related declines in cognitive flexibility(De Rosa, Hasselmo, & Baxter, 2001; Ebert & Anderson, 2009; Emery et al., 2008; Hasselmo, Bodelón, & Wyble, 2002). Overall these findings suggest that normal aging involves typical declines in cognitive flexibility which is crucial to one's ability to adaptively navigate the dynamic environments.

Indexes of Cognition - Vagal Tone and Heart Rate Variability

At the core of brain-body connectivity is the vagus nerve, a nerve associated with the communication between the autonomic nervous system (ANS, the body) and the central nervous system (CNS, the brain) and plays a significant role on regulating unconscious processes in varied tasks as heart rate (Appelhans & Luecken, 2006; Thayer & Sternberg, 2006; Warner & Cox, 1962). Upon the management of the vagus nerve, the two parts of the autonomic system – the parasympathetic and sympathetic nervous system – function synergistically to flexibly regulate the body, i.e., the two branches interacted together to increase or decrease one's heart rate while running or lying

(Akselrod et al., 1981; Rodrigues & Ewing, 1983). Indeed, it is well appreciated that what is good for the heart throughout life is also good for the mind. The vagus nerve decelerate it’s influence and adjust vagal tone through neurotransmitter acetylcholine (Howland, 2014). Vagal activities results in various effects, including heart rate reduction (Obrist, Wood, & Perez-Reyes, 1965). Vagal tone, which quantifies average traffic in cardiac vagal nerves, determines mean heart rate HR (Malik & Camm, 1990), has been associated with Cardiovascular states and longevity in the general population. By virtue of its influential impacts on heart rate, previous studies have suggested that vagal tone can be estimated by examining indexes of heart rate variability like RMSSD (Porges, Doussard-Roosevelt, & Maiti, 1994). RMSSD – Root mean square of the successive differences is one of the main time-domain tools used to assess HRV (heart rate variability), the successive difference being neighboring RR intervals (beat to beat differences in instantaneous heart rate). A number of documented studies have suggested RMSSD as the most common way to analyze HRV(Buchheit et al., 2010; Stanley, D’Auria, & Buchheit, 2015; Tarvainen, Ranta-Aho, & Karjalainen, 2002; Vesterinen et al., 2013).

Both vagal tone and HRV declined during healthy aging(Thayer, Yamamoto, & Brosschot, 2010). Previous studies have suggested that cognitive flexibility is promoted by increased HRV, via increased vagal tone(Thayer, Hansen, Saus-Rose, & Johnsen, 2009). Diminution in the vagal tone and HRV are associated with age-related cognitive impairment in the elderly. Previous research (Kim et al., 2006) suggested that decreased HRV is a potential role of cognitive impairment and cognition are regulated via the vagus nerve. Plausibly, individuals with higher HRV and a high level of vagal tone would have higher cognitive ability in old age(Hansen, Johnsen, Sollers, Stenvik, & Thayer, 2004).

Controlled Slow Breathing and Heart Rate Variability

It is well appreciated that the heart rate increases while inhaling, and vice versa while exhaling(Conrad et al., 2007).The impacts of respiratory state on cardiovagal activity is considered to be far more significant(Lehrer et al., 1997; Tonhajzerova, Mokra, & Visnovcova, 2013). Investigations into physiological effects of slow breathing toward a rate of 6 breaths per min demonstrated that slow breathing produce numerous health benefits and have significant physiological effects on the respiratory, cardiovascular, cardiorespiratory and autonomic nervous systems, especially on HRV (Van Diest et al., 2014).Key findings also suggest that in comparison with typical breathing, controlled slow breathing (particularly at around 6 breaths per min) contributes to an increase in fluctuations of both blood pressure and heart rate(Pramanik et al., 2009).

Heart rate, which varies from person to person, is the speed of the heartbeat assessed by the number of beats of one's heart per minute(Shen, Tompkins, & Hu, 2002). Our heart beats with fluctuation rather than immutably(Akselrod et al., 1981; Kobayashi & Musha, 1982). The instantaneous heart rate can be measured on an ECG recording as the time between beats: the R-R interval(Berger, Akselrod, Gordon, & Cohen, 1986). Fluctuation of R-R intervals is a physiological occurrence known as HRV (Heart Rate Variability), largely a product of parasympathetic and sympathetic nervous system activity which is considered as a qualitative index of "Sympathovagal balance", reflecting the weight of parasympathetic versus sympathetic autonomic control. Studies (Hayano et al., 1994) also reported that slow breathing augmented vagal power by entraining vagally induced cardiac resetting to the phase of respiration. It has also been shown that during controlled, slow, deep breathing, the respiratory phase modulation of sympathetic activity is stronger(Oneda, Ortega, Gusmao, Araujo, & Mion Jr, 2010). A wealth evidence in previous studies have demonstrated that controlled slow breathing contributes to increased HRV, which has been associated with better cognitive performance, and this is particularly significant when respiration slows down, particularly at around 6 bpm(Spira, Chen-Edinboro, Wu, & Yaffe, 2014).

HYPOTHESES

As outlined above, a growing body of research suggests that vagal tone is an important aspect of the communication between the body and the mind. The importance of vagal tone and HRV is also highlighted in cognitive neuroscience, suggesting that increased HRV and a higher level of vagal tone may in fact benefit the cognition. However, studies also showed that both the vagal tone and HRV decline during normal aging, which could contribute to an impairment in cognitive ability, particularly the cognitive flexibility. Moreover, ample studies indicate that during controlled, slow, deep breathing, there is a tendency for increased HRV at particularly 6 bpm, which is associated with promoted cognitive performance(Spira et al., 2014; Yadav & Mutha, 2016), suggesting that non-invasive approaches like slow breathing, may be used to stimulate the vagus nerve (Chapleau & Sabharwal, 2011) through increasing HRV. Thus, the use of controlled slow breathing techniques seems to be potentially an effective means of optimizing physiological parameters that occurs to be associated with health and longevity(Brown & Gerbarg, 2009).

In alignment with these findings, we aimed at examining the potential effect of controlled slow breathing on cognitive flexibility, based on previous work from our laboratory and from other which have suggested that breathing impacts on the cardiovascular system and the body and the mind are integrated in the brain at the resolution of a heartbeat (Li, Swallow, Chiu, De Rosa, & Anderson, 2018) Hence, the objectives of the current study were to (1) investigate whether HRV increased after controlled slow breathing (6 bpm) in both old and young; (2) evaluate the improvement of task performance if HRV increased in both old and young.

Given this background of empirical and theoretical work, we hypothesize that controlled slow breathing (6 bpm) contributes to an increase in HRV (Heart Rate Variation) among both young and older adults. Furthermore, the increased HRV will significantly improve aged adults' cognitive flexibility performance relative to young adults.

MATERIALS and METHODS

Primary Research Question

The primary research questions we would ask include: (1) Does controlled slow breathing (6 bpm) increase HRV in both young and old? (2) If so, does increased HRV benefit cognitive flexibility in both young and old?

Study Design

To examine if controlled deep breathing in older adults can help combat age-related decline in the ability to flexibly shift one's attention we carried out the breathing and attention tasks and investigated the task performance among young and older adults. We elicited adaptive responding using a paired-associate image discrimination task in which participants must resolve proactive interference, that is, they must overcome prior learning that conflicts with a current situation(Dulas & Duarte, 2016). Our laboratory has used both the rodent and human versions of this task successfully in the past(Caplan, McIntosh, & De Rosa, 2006; De Rosa & Hasselmo, 2000). This is a computer-based, counterbalanced, multiple-variable experiment mainly containing 16 parts as follows.

(1)**a controlled breathing practice**, in which participants are asked to learn inhaling and exhaling according to a high-pitched sound and a low-pitched sound, respectively; (2) **an untimed attention task practice**, during which participants are asked to learn an image discrimination task through trial and error. For each trial, two images will fill up the left and right side of the screen. One of them will be the target image, whilst participants are supposed to find the target out on their own through trial and error based on the "Ding" sound indicating the target image and the "Buzz" sound indicating the wrong image respectively. They are allowed to repeat the unlimited attention practice on their own pace as many as they want; (3) **a timed attention task practice**, during which participants are asked to respond correctly at full speed; (4) a **baseline breathing run,** during which participants are asked to breath spontaneously; (5) **an attention task A/B run** including two versions – version A and version B, participants are designated to different versions randomly according to their digital number respectively (version A for odd number and version B for even number); (6) **a sham breathing run**, which will serve as a control. This will allow within-subjects comparisons of the task performance under spontaneous breathing and under controlled slow breathing. It should be noted that the cognitive flexibility task can be administered repeatedly with novel images on each iteration, with participants task performance remaining robust over repetitions of the task. (7) **an attention task A/B run**; (8) either **a response reversal (RR) task or a proactive interference (PI) task** from the A/B pair just prior; (9) **a controlled slow breathing run**, participants are asked to breath at around 6 breaths per min according to the two sounds instruction; (10) **an attention task A/B run**; (11) **either a RR task or PI task** from the A/B pair just prior; (12**) a sham breathing run**; (13**) a controlled slow breathing run**; (14) **a sham breathing run**; (15) **a controlled slow breathing run**; (16) **a baseline breathing run**.

Participants

Thirty participants (14 young (age 18-35 year) and 16 older adults (age over 60 year)) were recruited via a Cornell University online system and through advertisements.

A total of 10 participants (Young=3, Old=7) were excluded. Seven were excluded due to failing to complete the task; two were excluded in virtue of failing to breath according to the instruction at around the correct rate under each task; one was excluded due to low heart phase data quality. The final sample consisted of **20** subjects (11 young, aged 18 - 35 years; 9 old, aged over 60 years). All had normal or corrected-to-normal vision, and normal or corrected-to-close-to-normal hearing, were right handed, reported no history of neurological or psychiatric diseases, and given consent forms according to a protocol approved by the ethics committee of the Cornell University. The key exclusion criteria were as follows: non-English speaker, a history of neurological disorder including seizures, strokes, concussion, serious head injury or other severe brain-related issues; currently taking medications that affect the cognition, currently using non-prescription drugs such as marijuana or methamphetamine; colorblind; unable to make responses on a computer keyboard or touchscreen.

Task and Procedure

Participants were asked to learn a controlled breathing task and an image discrimination task through the breathing and attention tasks. In this paradigm, the participants were instructed to try and align their breathing to two sounds, inhaling at a high-pitched sound and exhaling at a low-pitched sound. There were three breathing conditions in this task: baseline breathing (BB), sham breathing (SB) and controlled slow breathing (CB).

Under each breathing condition, a pair of images were presented in front of the participants and they responded by pressing the button "Left" and "Right" which was indicated on the keyboard with stickers. Correct responses are rewarded with a "Ding" sound, whereas incorrect responses are accompanied with a "Buzz" sound. There were two experimental versions of the attention task – version A and version B, and a practice version – Version C in the task. Participants first learn a baseline stimulus pair containing 2 sets of images (e.g. '**A+/B-' and 'C+/D-'** for run 1**, 'E+/F-' and 'G+/H-'** in version C**,** where **'+'** indicates rewarded target stimulus and **'–'** indicates a non-rewarded distractor stimulus) through trial and error and practice them with unlimited time on their own pace. They were allowed to repeat the practice run as they want. After this, participants advanced to an attention task practice at full speed as accurately as possible. People who cannot perform and complete the attention task practice at full speed will be notified that they have finished the task and then be excluded later. These stimulus pairing are schematically represented in figure 1.

Practice Run	Version C	Controlled breathing practice	
		An untimed attention task pratice	
		Timed attention task practice	
Experimental Run	Version A/B	Baseline Breathing (BB)	
		Attention task A/B run	Novel learning trials
		Attention task A/B run	
		Sham Breathing (SB)	
		Attention task A/B run	PI trials or RR trails
		Either PI or RR from the AB pair just prior	
		Controlled Slow Breathing (CB)	
		Attention task A/B run	PI trials or RR trails
		Either PI or RR from the AB pair just prior	
		Sham Breathing (SB)	
		Controlled Slow Breathing (CB)	
		Sham Breathing (SB)	
		Controlled Slow Breathing (CB)	
		Baseline Breathing (BB)	

Figure 1 A picture of the breathing and attention task

Those who became proficient in indicating the target of the baseline pair and successfully complete the attention task practice advanced to a testing phase in version A or version B. Within Version A/B, there are 3 trails under 3 different breathing conditions respectively. Each trial has 2 experimental runs and 2 pairs of images within each run. Participants were randomly assigned to different versions according to their digit number, odd with version A and even with version B.

First, the participants were asked to finish 2 attention task A/B runs under baseline breathing (BB) condition, this task tested participants' cognition ability on novel learning based on 2 pairs of images (e.g. '**A+/B-' and 'C+/D-'** for run 1**, 'E+/F-' and 'G+/H-'** for run 2**,** where **'+'** indicates rewarded target stimulus and **'–'** indicates a non-rewarded distractor stimulus). After taking a short break, participants moved to a sham breathing task and were asked breath at the instructed rate according to, then they advanced to another 2 experimental runs, consisting of one attention task A/B run (e.g., '**A+/B-' and 'C+/D-' for run 1**) and interleaved trials of two types based on the previous stimulus: **PI** (proactive interference) **trials** (e.g., '**E+/A-' and 'C+/D-' for run 2**) in which the target from the baseline (**A+/B-)** is now the distractor (**E+/A-),** paired with a new stimulus target**,** or **RR** (Response Reversal) **trials** (e.g., '**A-/B+' and 'C-/D+'**) in which the rewarded target stimulus from the baseline (**A+/B-; C+/D-**) becomes the non-rewarded distractor stimulus (**A-/B+; C-/D+**). To succeed in PI trials participants must overcome past learning about a former target stimulus; thus, proactive interference is elicited. For instance, image A is a target in the context of pairing with image B but a distractor in the context of pairing with image C. The stimulus pairing in version A/B are schematically represented in figure 2.

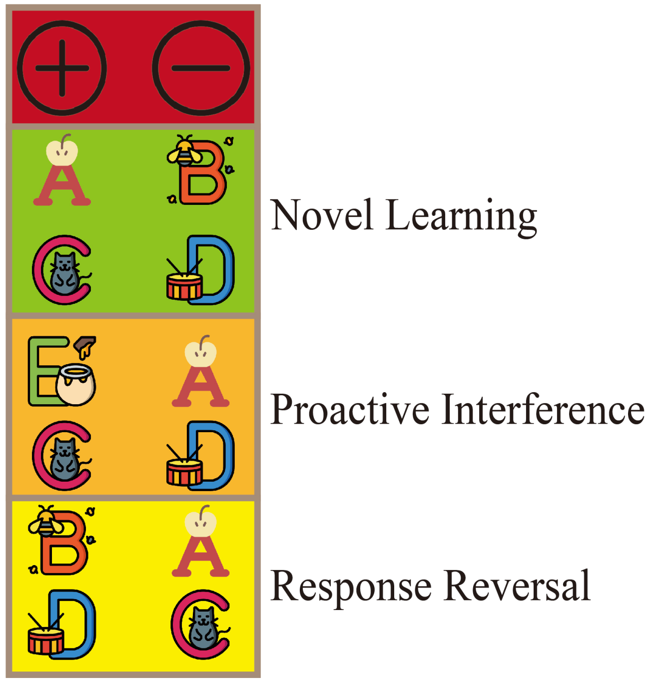


Figure 2 A TESTING PHASE IN VERSION A/B; SCHEMATIC OF IMAGE PAIRS. COLORS/LETTERS INDICATE DIFFERENT IMAGE STIMULI. + INDICATES TARGET, - INDICATES DISTRACTOR.

The primary outcome measure of attention task performance is accuracy, the percentage of trials of each type in which the participant selected the correct target stimulus. Lower accuracy in the PI trials compared to the Novel trials suggests that participants are experiencing proactive interference, biasing them toward inappropriate retrieval of past experience rather than adaptively responding to a new scenario. The primary outcome measure of breathing task performance is RMSSD and Mean Heart Rate from ECG and PPG for young, RMSSD and Mean Heart Rate from PPG for old.

Statistical Methods

We collected physiological data from all the breathing and attention task runs for this whole experiment.

First, we recorded baseline ECG, PPG and respiration using the Biopac-MP150 system (Biopac Inc., New York, USA) during baseline breathing, sham breathing and slow breathing. Real-time heart phase detection results were plotted along with the ECG and PPG amplitude and visually inspected and manually artifact corrected using Kubios HRV Premium 3.1.0 (https://www.kubios.com/hrv-premium/). Data analysis was done IN Graphpad Prism 7.0 (Graphpad Software，Inc.). One participant was excluded as the plotted detected peaks were apparently off. Two participants were excluded in virtue of failing to breath according to the instruction at around the correct rate under each task. Seven participants were excluded due to failing to complete the task. The physiological information of all participants under different breathing conditions are shown in figure 3.

Phsiological Inforamtion for Older and Young Adults under Different Breathing Conditions

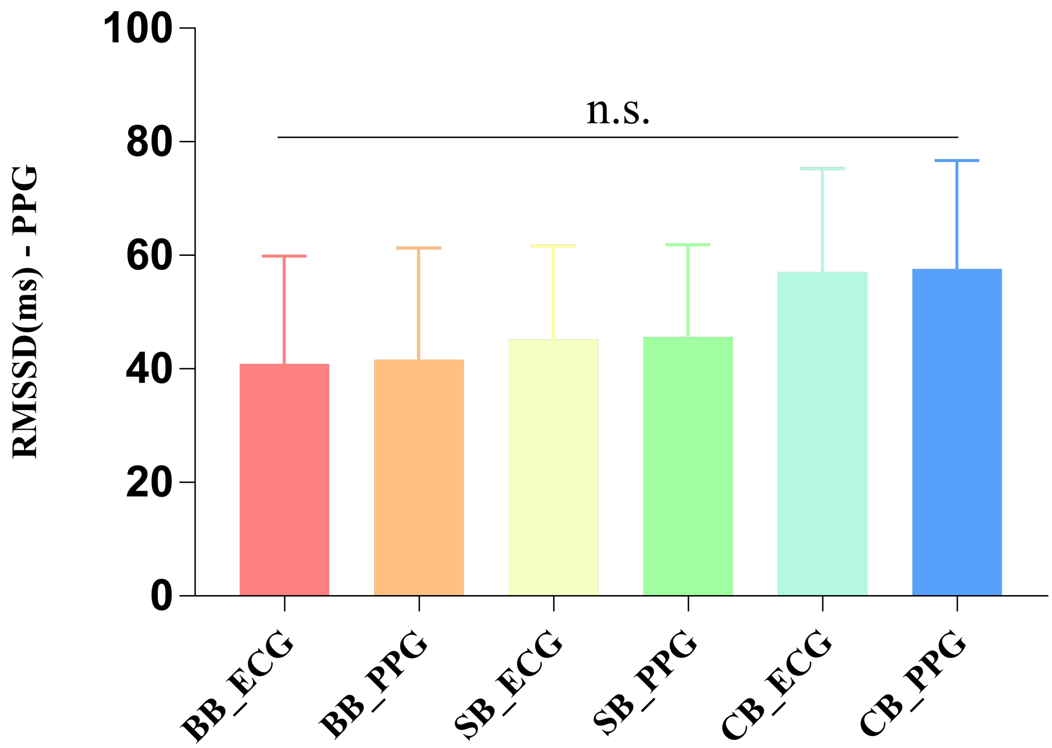
Sub ID	BB（Baseline Breathing）				SB (Sham Breathing)				CB (Controlled Breathing)			
	RMSSD(ms) - ECG	HR - ECG(bpm)	RMSSD(ms) - PPG	HR - PPG(bpm)	RMSSD(ms) - ECG	HR - ECG(bpm)	RMSSD(ms) - PPG	HR - PPG(bpm)	RMSSD(ms) - ECG	HR - ECG(bpm)	RMSSD(ms) - PPG	HR - PPG(bpm)
Young_101	12.011	75.386	12.056	75.646	46.112	71.567	46.117	71.542	39.203	69.486	39.022	69.498
Young_102	30.477	78.639	28.608	78.097	50.127	78.137	50.248	78.118	30.607	79.773	31.757	79.783
Young_103	12.572	73.621	13.208	73.548	39.174	74.846	40.504	74.985	63.109	69.753	63.546	69.722
Young_104	63.325	65.655	61.519	67.881	50.836	67.87	52.959	68.162	94.794	63.877	98.132	63.841
Young_105	54.039	70.024	57.755	70.592	30.723	75.436	31.314	75.427	56.43	68.511	57.217	68.578
Young_106	43.989	69.849	42.412	69.961	31.243	75.745	30.551	75.501	41.261	74.895	41.986	74.936
Young_107	51.432	66	54.038	66.158	79.211	72.027	79.152	71.993	53.809	68.233	54.186	69.354
Young_108	64.489	67.926	67.412	68.767	61.278	76.004	61.651	76.046	79.5	68.031	80.161	68.028
Young_109#	EXCLUDED											
Young_110	52.35	83.843	53.884	82.663	29.904	88.413	30.139	88.327	37.943	86.199	37.28	86.196
Young_111	36.214	64.462	37.605	64.574	39.424	65.884	41.189	65.906	59.11	65.486	60.092	65.739
Young_112	19.694	66.368	18.404	67.57	60.99	79.572	59.724	80.333	64.57	69.819	64.307	72.821
Young_113	39.684	68.992	39.793	68.547	28.758	72.251	28.264	72.289	37.752	74.771	37.758	74.709
Young_114#	EXCLUDED											
Old_200#	EXCLUDED											
Old_201			36.44	124.77			38.851	87.882			48.642	107.79
Old_202			41.644	72.047			50.573	90.35			50.324	91.505
Old_203			66.917	64.921			36.968	76.974			65.3	64.614
Old_204			28.59	58.939			30.081	61.692			47.064	63.358
Old_205			7.079	67.992			28.217	72.516			91.319	70.13
Old_206			28.733	85.325			65.226	88.547			90.284	90.151
Old_207#	EXCLUDED											
Old_208#			88.834	112.71			106.61				131.01	110.96
Old_209#	EXCLUDED											
Old_210#	EXCLUDED											
Old_211			33.242	129.67			120.57	84.14			144.98	101.15
Old_212			49.592	100.21			79.837	98.225			145.61	103.27
Old_213#	EXCLUDED											
Old_214			4.826	91.533			46.543	91.433			90.851	87.522
Old_215			1.914	78.023			43.704	93.618			91.389	91.322

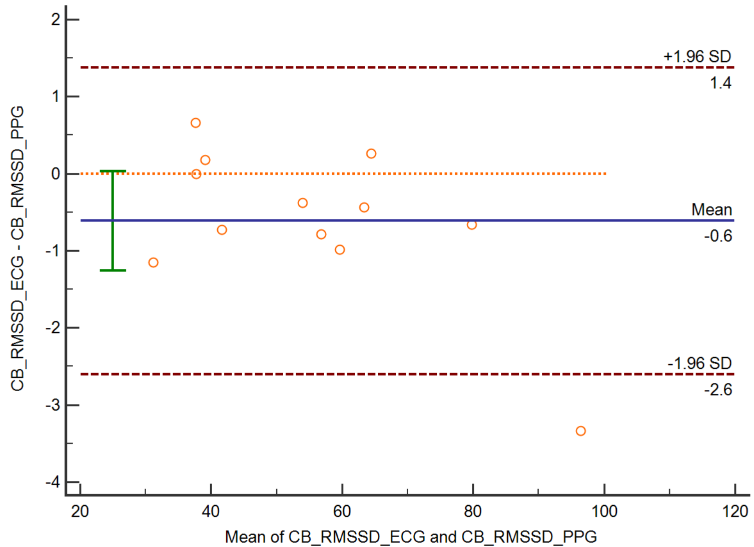
N=30 (Young=14, Old=16); Excluded=7 (Young=2, Old=5)

People failed to complete the task

Figure 3 Physiological information for Participants under Different Breathing Conditions

Based on the data we obtained above, we compared the consistency of the heart rate signals extracted by the PPG and ECG in young adults to determine how closely they align with each other using paired *t* test. According to the result, there is no significant difference between the ECG and PPG in BB, SB and CB ($p<0.05$), which could validate the validity of our study (figure 4). Since the difference between ECG and PPG is 0.75±0.56 (BB), 0.36±0.31 (SB), 0.56±0.32(CB), which was shown in table 1.





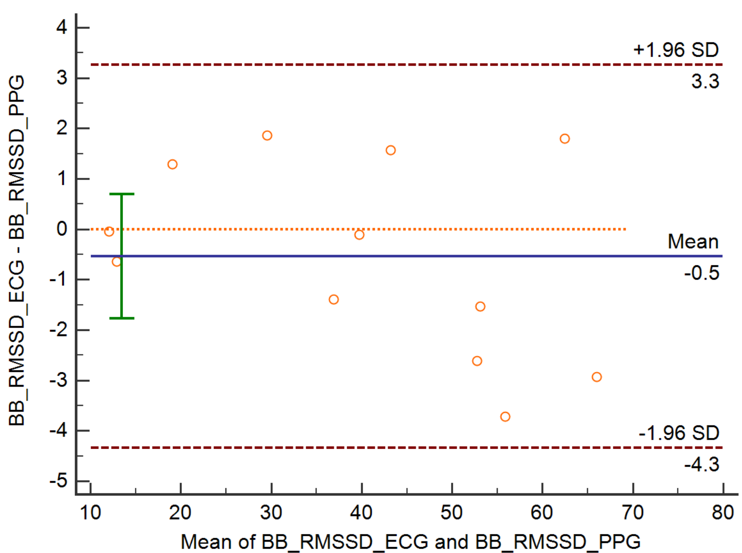
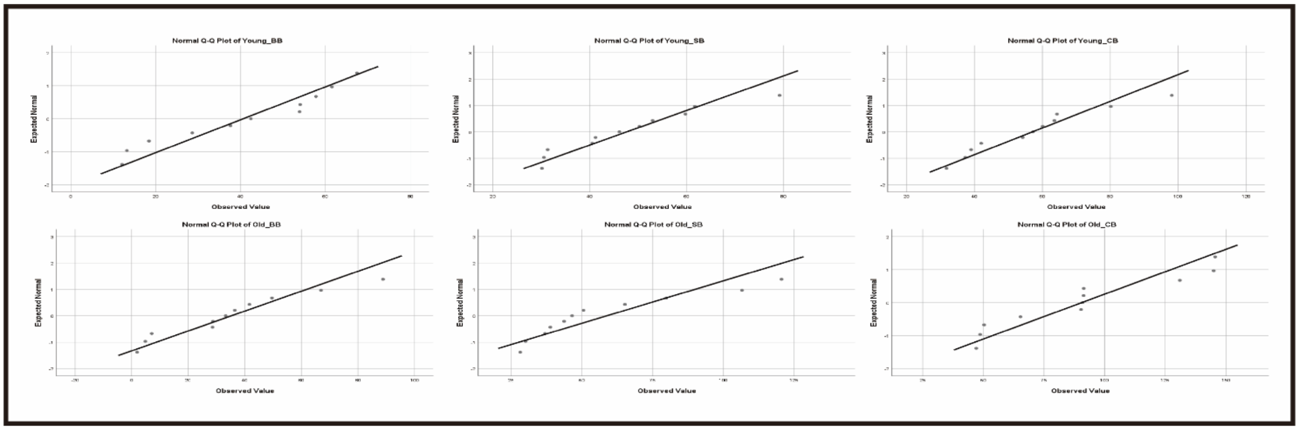


Figure 4 Consistence between ECG and PPG in young adults

Table 1 Consistence between ECG and PPG in young adults

	ECG	PPG	Paired *t*	*p*
Baseline Breathing (BB)	40.89±5.7	40.56 ± 5.5	1.335	0.2114
Sham Breathing (SB)	45.2±4.9	45.98±4.5	1.131	0.2845
Control Breathing (CB)	57.04±5.5	55.45±5.7	1.779	0.1056

First, the normality of numerical continuous data was tested: if the data was normal distribution, then *t*/paired *t* test is performed to check the difference between the two groups; if the data was non-normal distribution, then non-parametric test is used. Bonferroni correction is used for multiple comparison of the mean between difference groups (＞2). Bilateral test was performed. $p<0.05$ means the difference is significant (figure 5).



Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Young_BB	0.200	11	.200*	0.916	11	0.286
Young_SB	0.130	11	.200*	0.932	11	0.429
Young_CB	0.175	11	.200*	0.938	11	0.494
Old_BB	0.132	11	.200*	0.938	11	0.496
Old_SB	0.241	11	0.073	0.857	11	0.053
Old_CB	0.219	11	0.147	0.881	11	0.106

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Figure 5 Results of normality test

According to the result of Kolmogorov-Smirnov and Shapiro-Wilk test, all the data sets are the normal distribution (p>0.05).

RESULTS

Comparison of HRV between young and older adults under BB

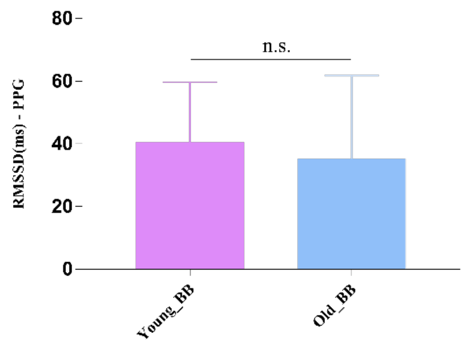


Figure 6 Comparison of HRV between young and older adults under BB

As shown in the Fig.6, there is no significant difference between the Baseline Breathing data of the two groups (p>0.05).

Comparison of HRV in young adults between BB, SB and CB

Table 2 Comparison of HRV within each group

	Baseline Breathing	Sham Breathing	Control Breathing	paired t	P^*
Young	40.56 ± 5.5	45.98±4.5	55.45±5.7	0.8037	0.4386[a]
				2.419	0.034[b]
				1.684	0.1203[c]
Old	35.25 ± 8.0	58.83±9.4	90.62±11.1	2.608	0.0261[a]
				4.502	0.0011[b]
				5.002	0.0005[c]

[a] Comparison between BB and SB; [b] Compartison between CB and BB;
[c] Comparison between SB and CB
[*] B Bonferroni correction for multiple comparison, thus, α=0.05/3=0.0167. P<0.0167 represents significant difference.

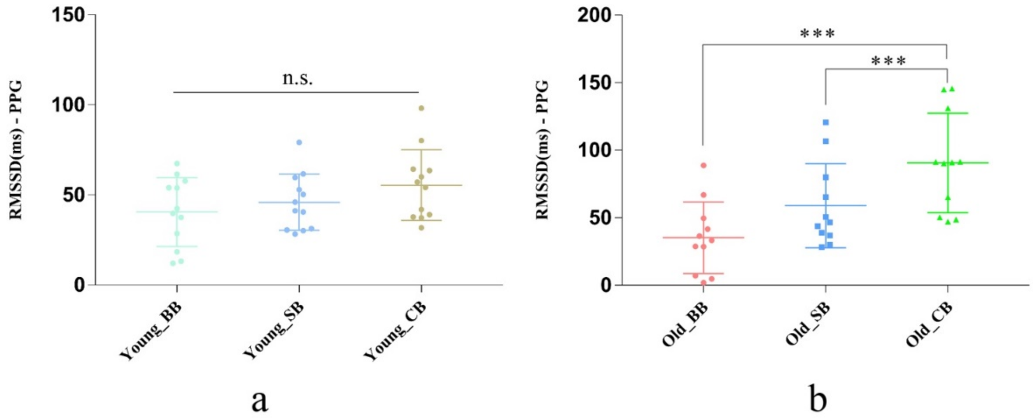


Figure 7 Comparison of HRV in young adults between BB, SB and CB

Among young participants (figure 7a), there is no difference of the HRV between BB vs SB, BB vs CB and SB vs CB.

Among old participants (figure 7b), compared to the baseline breathing, even though HRV in SB seems higher than BB, there is no significantly difference between SB and BB. However, HRV in CB is significantly higher than both BB and SB. (CB vs. BB, p=0.0011; CB vs. SB, p=0.0005) (shown in table 2).

Comparison of HRV between young and older adults under BB

Table 3 Comparison of the increasing level of HRV within each group

	Young	Old	*t*	*P*
PPG_(CB-BB)	14.9 ± 6.2	55.36 ± 12.3	3.02	0.0065
PPG_(SB-BB)	5.427 ± 6.8	23.58 ± 9.0	1.627	0.1187

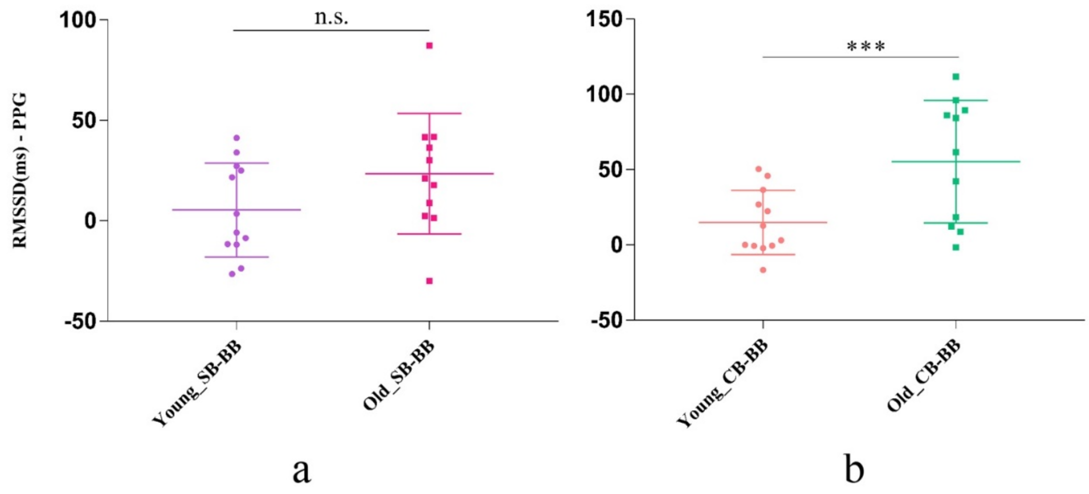


Figure 8 Comparison of the increasing level of HRV from BB to CB between young and old

For older participants, the increase of HRV from BB to SB seems bigger than that of young, but the difference is not statistically significant ($p>0.05$) (figure 8a).

For older participants, the increase of HRV from BB to CB is significantly higher compared to that of young ($p<0.01$) (figure 8b) (shown in table 3).

Discussion

In several studies respiration patterns have been manipulated in an attempt to influence ANS functioning(Martarelli, Cocchioni, Scuri, & Pompei, 2011). Ample previous research on deep breathing have reported a plethora of beneficial effects associated with physical health, mental health and cognitive performance(Gerritsen & Band, 2018) However, these studies are mainly carried out in a healthy young population, suggesting how operant respiratory intervention benefits young adults, not much literature has been devoted to revealing the reported benefits on aged people who are undergoing age-associated shifts. We examined how HRV is affected by different breathing patterns (BB, SB and CB) among both young and older adults.

Consistent with prior findings(Zhang, 2007),, our study showed a similar HRV range for both young and older adults. However, given the numerous evidence indicating that HRV of young was significantly larger than old(Boutcher & Stocker, 1996), we would have expected significantly greater HRV in young adults compared to older adults under baseline breathing(Craft & Schwartz, 1995). A body of previous research revealed that elderly participants showed lower HRV compared to the young participants when supine. However, the increase of HRV in young adults was significantly higher than that of older participants when standing up(Simpson & Wicks, 1988). Yet our study found that even HRV in the young adults seems slightly greater than that of old, but there is no significantly difference between the two. This inconsistence might be due to a relatively small sample size of our study.

Manipulation of respiratory patterns across studies have demonstrated how controlled slow breathing (particularly at around 6 breaths per minute) physically and tonically stimulate the vagus nerve via significantly increasing the HRV, associated with numerous health outcomes(Conrad et al., 2007; Hirsch & Bishop, 1981; Lee et al., 2003; Lehrer & Gevirtz, 2014; Montgomery, 1994; Mortola, Marghescu, & Siegrist-Johnstone, 2015; Pal & Velkumary, 2004; Perciavalle et al., 2017; Tavares et al., 2017;

Van Diest et al., 2014). Thus, we have examined the changes of HRV among young and older adults under different breathing patterns. According to prior findings(Zhang, 2007) , the increase of HRV should align with the age, suggesting that young adults should have much greater increase of HRV compared to older adults. Unsurprisingly, some of our findings were partly aligned with these prior studies, that is, the stimulation of breathing indeed increased the HRV in young people (i.e, there was an increase from BB to SB, an increase from SB to CB, and an increase from BB to CB, but the difference was not statistically significant) . However, in the present study, there were somewhat surprising findings demonstrating that in comparison with young adults, there was significantly increase of HRV under controlled slow breathing (at around 6 bpm) compared to that of baseline breathing. Unlike the direct experience evoked here, our results suggested that compared to young, controlled slow breathing showed better influence in older adults, which is consistent with a previous study in particular examining the effects on aged people(Bretherton et al., 2019).

Furthermore, we compared the increasing level of HRV between young and old, found that in comparison of young participants, the increase of HRV from BB to SB of older participants is slightly greater, but the difference is not significant. On the other hand, the increase of HRV from BB to CB in older adults is significantly greater than that of young.

Even previous evidence indicated that 20-year-old people often show a swing of 5-10 points between the high and low points in their heart rates, while people over 50s often show changes of only 3-5 beats(Seeherman & Morris, 1991). They also argued that people who are more physically active show a wider range between their maximal and minimal heart rate(Haskell et al., 2007). Our study showed a quite different result. We suggested that this could be due to the different starting HRV of young and older adults(Clancy et al., 2014). To be more specifically, with the decline of HRV during healthy aging, the operant respiratory intervention could be particularly effective in older adults compared to younger participants (Abhishekh et al., 2013; Barantke et al., 2008; Felber Dietrich et al., 2006; Umetani, Singer, McCraty, & Atkinson, 1998).

For example, previous studies showed that the average maximum heart rate for young people aged 20 years old is about 200bpm, however, for people aged over 70, the average maximum is around 150 bpm. It is apparently that the two aged groups have different standard for health condition and different threshold for health assessment criteria ("Target Heart Rate and Estimated Maximum Heart Rate,"). As people exercise, it is highly possible that for a 20-year-old young man whose normal heartbeat is pretty fast, one hour jogging may enhance his heart rate to 130 bpm from 100 bpm, yet for a 70-year-old senior, the same amount of exercise may cause his heart rate increasing from 75 bpm to 150 bpm. The threshold is different, thus even the same stimuli could have different effects.

An alternative explanation is, with the decrease of HRV during normal aging, the relatively lower level of older adults also suggested that there is a larger room for improvement in comparison of young people who have already had pretty high HRV. Thus, it is not difficult to comprehend that for a test with a full score of 100 points, it’s much easier for those who scored 30 points at the beginning to achieve 60 points compared to those who have already had 95 points who aimed to gain a full score of 100 points. Because the baseline level for young people are already very high, the space for improvement for them is not as large as that of aged people who has a relatively larger room for improvement. Thus, the level of stimulation required to increase HRV of young is much higher than that of the elderly.

Although there has been a body of research indicating that HRV declines across the lifespan(Abhishekh et al., 2013; Barantke et al., 2008; Felber Dietrich et al., 2006; Umetani et al., 1998). Increasing evidence have suggested that HRV is malleable under a variety of conditions, such as controlled slow breathing at around 6 breaths per min as our study demonstrated. There is accumulating evidence suggesting that VNS has been shown to affect cognitive functioning, for example memory consolidation and recognition(Clark, Naritoku, Smith, Browning, & Jensen, 1999; Ghacibeh, Shenker, Shenal, Uthman, & Heilman, 2006; Vonck et al., 2014). Traditional implanted VNS approaches have been proven to be useful and approved for treating major depressive disorder and epilepsy in adults(Sackeim et al., 2001). However, due to its invasive nature, technical complications and side-effects (i.e., pain) potentially simpler and safer therapies are of interest(Johnson & Wilson, 2018).

As such, our study suggested that there appears to be potential for use of controlled slow breathing techniques as an effective means of maximizing HRV, increasing vagal activity as well as augmenting vagal power especially in older adults. Importantly, our findings point to the achievability of this simple non-invasive vagal nerve stimulation. With simple practice it is easy to be used by most individuals especially for those who are less likely to do physical exercises, and could even be useful for a wider range of immobile aged people as well. Importantly, there is not any documented adverse yet (Russo, Santarelli, & O'Rourke, 2017).

One important limitation in our study is the small sample size. We predict that with larger sample size, there might be a larger difference of HRV between young and older adults under baseline condition. In conclusion, it appears possible that this nVNS approach significantly improves the HRV through regulating the vagus nerve and thus results in a higher level of vagal tone particularly in elder people, but further research is needed to explore the biological mechanism behind this influence and investigate how to maximize its health-promoting function.

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